ENZYMATIC SYNTHESIS OF RADIO-LABELED SELENOCYSTINE FROM LABELED SERINE AND SELENIDE

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SUMMARY

Selenocysteine can be formed enzymically from O-acetylserine and sodium selenide by O-acetylserine sulfhydrylase. The preparation of O-acetylserine from serine and its conversion to selenocystine are described. These reactions can be used to synthesize [^{14}C], [^{3}H], or [^{75}Se]selenocystine.

Key Words: Selenocystine, selenocysteine, O-acetylserine, selenide, serine, O-acetylserine sulfhydrylase.

INTRODUCTION

O-acetylserine sulfhydrylase from Salmonella typhimurium catalyzes the formation of cysteine from O-acetylserine and hydrogen sulfide (1). The ability of hydrogen selenide, the selenium analogue of hydrogen sulfide, to undergo this reaction had been suggested (2) and recently has been reported to occur in higher plants (3). The following report documents the formation of selenocysteine with the bacterial enzyme and its use in the synthesis of radio-labeled selenocystine.

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EXPERIMENTAL

Materials

[G-^{3}H]L-Serine and [U-^{14}C]L-serine were purchased from the International Chemical and Nuclear Corporation, Irvine, CA. [^{75}Se]selenous acid was purchased from New England Nuclear Corp., Boston, Mass. All reagents were of common laboratory grade. *S. typhimurium* O-acetylserine sulfhydrylase was a kind gift from Dr. N. Kredich.

NaHSe was generated by acidifying, with H_3PO_4, the reaction product of elemental selenium and aqueous KBH_4 and transferring the H_2Se formed to an anaerobic sodium hydroxide solution using a flow of argon gas. If the system was not anaerobic the formation of a red product (Se°) in the NaOH solution was observed. If ^{75}Se-labeled NaHSe was required, [^{75}Se]selenous acid was added to the selenide solution. [^{75}Se]selenous acid equilibrates rapidly with the unlabeled selenide.

Thin layer chromatography was performed on silica gel sheets in chloroform-ethanol-glacial acetic acid-water, 50:32:10:8 according to Kredich and Tomkins (1). Amino acid analysis of the selenocysteine derivatives was performed on the long column (0.9 x 65 cm) of a Beckman 121 Analyzer using the buffer systems of Spackman et al (4). Radioactivity was measured by scintillation spectrometry in either Aquasol (New England Nuclear, Inc.) or in a mixture containing 2 parts ethanol and 8 parts 0.79% (wt/vol) PPO (2,5-diphenyloxazol) in toluene.

RESULTS AND DISCUSSION

Preparation of O-acetylserine

O-acetylserine was prepared using radioactive or non-labeled serine using the minor variations of published procedures (5,6), proposed by Dr. Kredich. Serine was dried in a tube and 0.5 ml of glacial acetic acid, saturated with HCl gas, was added. The tube was capped and allowed to incubate overnight at room

temperature. The tube was heated (30°C-40°C) under a stream of nitrogen gas until dry. Thin layer chromatography of the residue (Figure 1) shows the almost quantitative yield of O-acetylserine (approximately 94%) which was derived in this case from [^{3}H]serine.

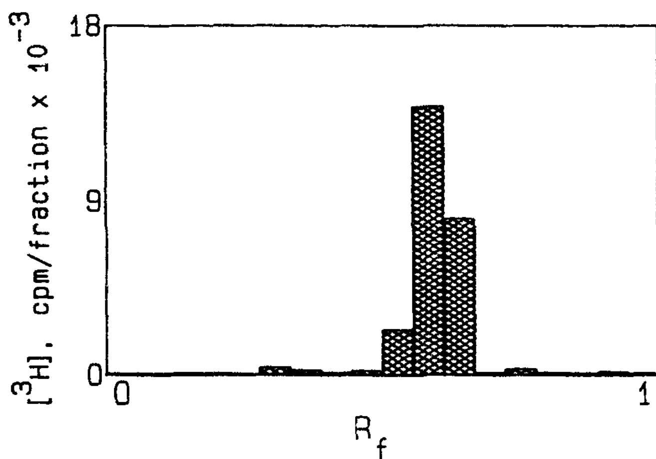

Figure 1. Thin layer chromatography of [^{3}H]O-acetylserine preparation.

Preparation of selenocystine

O-acetylserine was resuspended in 200 µl 1 M K phosphate (pH 7.2) and flushed with argon. 100 µl of selenide solution (0.2 N NaOH saturated with H_2Se) was added anaerobically along with 2 µl of O-acetylserine sulfhydrylase (4-20 units). After 45 minutes another 2 µl of enzyme was added. Glacial acetic acid (30 µl) was added 45 minutes later and the vial was flushed with argon to remove H_2Se. If [^{75}Se]selenide was used, a stopper fitted with an inlet and exit port was provided and the radioactivity released from the vial was captured in an alkaline peroxide solution.

The reaction mixture was adjusted to pH 1 with HCl and left exposed to the air for 1 hr. A small amount of red turbidity formed. The mixture was then placed on a 1.5 ml Dowex-50 X-8 (H^+) column and the adsorbed product eluted with 2 N NH_4OH. The first basic fractions were dried under argon with low heat (30-35°C). The dried product was resuspended in water and purified by thin layer chromatography (see Fig. 2), with the major contaminant being serine (R_f 0.3). The selenocystine(R_f 0.08) was eluted from the chromatogram with water, dried under argon (30-35°C) and stored in the cold.

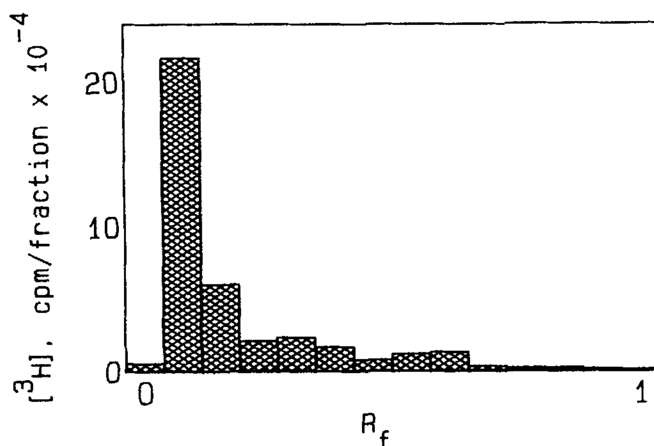

Figure 2. Thin layer chromatography of [^{3}H]selenocystine preparation.

Selenocystine synthesized from [^{3}H]serine was mixed with unlabeled selenocystine, reduced with $NaBH_4$ and alkylated with 2-bromoethanol. The alkylating agent was removed by placing the acidified (with HCl) reaction mixture on a 1 ml Dowex-50 X-8 (H^+) column and eluting the alkylated amino acid with 2 N NH_4OH. The radioactive fractions were dried and resuspended in 0.066 M sodium citrate buffer pH 2.2 (0.2 M in Na^+). This sample was subjected to amino acid analysis. Figure 3 shows the profile of the two ninhydrin positive and ^{3}H-containing peaks. The major peak which was eluted at the position of Se-hydroxyethyl-selenocysteine, contained 90.2% of the applied radioactivity. The smaller, leading

peak (12.3% of the ^{3}H) was likely an oxidation product of the alkylated selenocysteine. This oxidation has been described by Cone et al (7). The same experiment was performed with iodoacetamide as the alkylation agent with a recovery of 102% in a single ninhydrin positive and ^{3}H-containing peak corresponding to Se-carboxamidomethyl selenocysteine. The coincidence between the ninhydrin profile, which measured the added carrier selenocysteine, and the radioactivity profile, indicates that the radioactive product is in fact selenocystine.

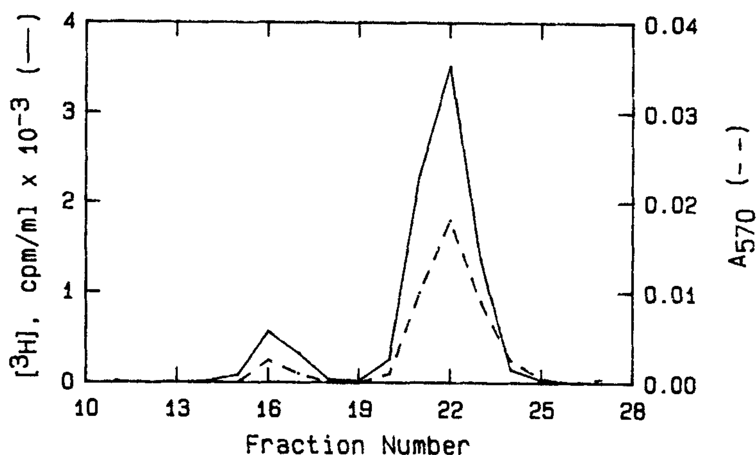

Figure 3. Amino acid analysis of Se-hydroxyethyl-selenocystine.

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